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| REPORT DOCUMENTATION PAGE | | READ INSTRUCTIONS BEFORE COMPLETING FORM |
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| 1. REPORT NUMBER AFIT/CI/NR 88-186 | 2. GOVT ACCESSION NO. | 3. RECIPIENT'S CATALOG NUMBER |
| 4. TITLE (and Subtitle) THE USE OF IONTOPHORETICALLY APPLIED ACYCLOVIR ON RECURRENT HERPES LABIALIS | | 5. TYPE OF REPORT & PERIOD COVERED MS THESIS |
| | | 6. PERFORMING ORG. REPORT NUMBER |
| 7. AUTHOR(s) LEWIS G. HUMPHREYS, Jr | | 8. CONTRACT OR GRANT NUMBER(s) |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS AFIT STUDENT AT: BAYLOR UNIVERSITY | | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS |
| CONTROLLING OFFICE NAME AND ADDRESS | | 12. REPORT DATE 1988 |
| | | 13. NUMBER OF PAGES 96 |
| MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) AFIT/NR Wright-Patterson AFB OH 45433-6583 | | 15. SECURITY CLASS. (of this report) UNCLASSIFIED |
| | | 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE |
| DISTRIBUTION STATEMENT (of this Report) DISTRIBUTED UNLIMITED: APPROVED FOR PUBLIC RELEASE | | |
| 17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) SAME AS REPORT | | |
| 18. SUPPLEMENTARY NOTES Approved for Public Release: IAW AFR 190-1 LYNN E. WOLAVER Lynn Wolaver 19 Aug 88 Dean for Research and Professional Development Air Force Institute of Technology Wright-Patterson AFB OH 45433-6583 | | |
| 19. KEY WORDS (Continue on reverse side if necessary and identify by block number) | | |
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# THE USE OF IONTOPHORETICALLY APPLIED ACYCLOVIR ON RECURRENT HERPES LABIALIS

A THESIS SUBMITTED TO THE FACULTY OF

BAYLOR UNIVERSITY

IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE

OF

MASTER OF SCIENCE

BY

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DALLAS, TEXAS

AUGUST 1988

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ABSTRACT

A randomized, double-blind placebo controlled study was undertaken to determine the efficacy of iontophoretically applied acyclovir in the treatment of herpes simplex labialis. Twenty-five patients ~~agreed~~ to participated in the study; however, only eleven actually reported any herpetic lesions. The patients were otherwise healthy individuals who gave a history of at least three episodes of herpes labialis in the past twelve months. With each episode, the lesions were photographed and classified ~~as~~ to size, location and stage.

All lesions were treated within twenty-four hours of onset. If a patient presented after twenty-four hours of onset the lesion was followed untreated. Also, if a patient presented with multiple lesions at the same time, one lesion was left untreated. Treatment consisted of a single iontophoretic application of acyclovir or the placebo, sodium bicarbonate. Healing of the lesions was monitored at several subsequent visits until complete. Clinical parameters statistically analyzed included change in lesion size twenty-four hours following treatment, time to the loss of lesion crust, and time to complete healing.

Statistical analysis revealed no differences among the groups when comparing initial lesion size and change in lesion size after twenty-four hours. There was no difference in duration of healing between the acyclovir-treated lesions and the placebo-treated lesions. However, both acyclovir and the placebo demonstrated some efficacy when compared to untreated lesions. It appears that the iontophoretic application of acyclovir provides only limited clinical benefit in treating herpes labialis.

Keywords: Antiviral agents, Ointments, Theses.(

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# ACKNOWLEDGMENTS

I would like to thank Dr. John Wright for his assistance in photographing the cytologic slides and his constructive remarks during the preparation of this manuscript. I would also like to thank Dr. Francisco Rivera-Hidalgo for his guidance throughout my residency. I am deeply indebted to Mr. Kong Huang for his patience and relentless assistance to me.

I would like to extend a special thanks to Dr. Terry Rees and Dr. William Hurt. Thank you for the excellent education I have received at Baylor. It has been both a privilege and honor to have trained under two "giants" in the field of periodontics.

Most of all, I would like to express my deepest gratitude to my wife, Alicia. Without her understanding nature and continuous support I would have fallen short of reaching the goals that I have achieved. Thus it is with utmost pleasure that I conclude my residency at Baylor by dedicating this manuscript to her.

## CHAPTER I

## INTRODUCTION

Recently there has been renewed interest in the herpes viruses not only in the scientific community but also among the lay public. There are now over fifty herpes viruses that have been identified, four of which are known to afflict mankind. They are: the cytomegalovirus, the Epstein-Barr virus, the herpes zoster (varicella zoster) virus, and herpes simplex virus type I and II. The cytomegalovirus infects the salivary glands and vital organs of infants and has been associated with Kaposi's sarcoma (Bhaskar 1985). The Epstein-Barr virus causes infectious mononucleosis and has been closely linked to Burkitt's lymphoma and nasopharyngeal carcinoma (Lynch, Brightman, and Greenberg 1984). The varicella zoster virus causes herpes zoster and chicken pox (Kaplan 1973). Herpes simplex I (HSV-I) is responsible for the majority of oral and pharyngeal herpes infections, keratoconjunctivitis, and herpes meningoencephalitis (Lynch et al. 1984; Bhaskar 1985). Herpes simplex II (HSV-II) is implicated in a majority of genital herpes infections and infections of the newborn (Evans 1982).

The herpes simplex virus is composed of three primary structural units: (1) a core of nucleic acid and associated proteins, (2) an icosahedral protein capsid, and (3) a lipid-containing envelope (Hicks and Terezhalmy 1979; Corey and Spear 1986a). The core consists of a linear, double-stranded DNA molecule with a molecular weight of 100 $X10^6$ daltons (Corey, et al. 1986a). There is considerable homology between HSV-I and HSV-II genomes with 50 percent of the nucleotide sequences being identical (Buchman et al. 1980). The DNA core is compactly folded and surrounded by the protein capsid. This process is facilitated by the core proteins which are specific for HSV-I and HSV-II. The capsid is made up of six distinct protein components which form 162 prismatic capsomeres (Hicks et al. 1979). The capsid, viral DNA, and core proteins are collectively referred to as the nucleocapsid.

The third component of the herpes simplex virus is the envelope. This is a two-layered structure which surrounds the nucleocapsid. A lipid-rich outer layer is derived from the biochemically modified inner lamella of the host cell nuclear membrane (Hicks et al. 1979). During viral replication, nucleocapsids are assembled in the nucleus of the infected cell and envelopment occurs as the nucleocapsids bud through the inner nuclear membrane into the cell's cytoplasm (Corey et al. 1986a). Once

inside the cytoplasm, the virions are transported through the endoplasmic reticulum and the Golgi apparatus to the cell surface and subsequently released. Embedded in the lipid bilayer of the envelope are several viral glycoproteins which mediate attachment of the newly formed virus to other host cells where viral invasion and replication can take place (Corey et al. 1986a).

Two types of infections occur due to the herpes simplex virus. They are: (1) PRIMARY infection, which occurs in patients without prior immunity to the virus, and (2) RECURRENT or SECONDARY infection, where circulating antibodies to the virus are present (Adam 1982; Straus et al. 1985). Upon initial exposure to the virus, the primary infection may be unapparent or it may have a clinically manifest course. It has been estimated that 90 percent or more of primary HSV-I infections and about 50 percent of primary HSV-II infections are undiagnosed because of the minimal nature of the signs and symptoms (Bolognese et al. 1976; Blincoe and Miller 1979). Symptomatic primary infections , because of a lack of immunity, are generally more severe than recurrent infections (Shafer, Hine, and Levy 1983). Primary infections with HSV-I usually occur in children, with the majority of cases occurring between the ages of one and four years (Straus et al. 1985). In contrast, primary infections with HSV-II occur most frequently in sexually

active individuals, with a peak incidence in the third decade (Corey et al. 1986b).

There is no specific site predilection for the two types of herpes simplex viruses. In general, HSV-I is the causative agent of oral infections and infections above the waist, while HSV-2 infection results in genital herpes and lesions below the waist. However, both viral subtypes can cause genital and oral-facial infections, which clinically are indistinguishable (Adam 1982; Corey et al. 1986a). In children, nearly all oral herpes infections are caused by HSV-I; however, in adults, as much as one-third of primary oral herpes may be caused by HSV-II (Adam 1982; Straus et al. 1985; Corey et al. 1986b). The majority of primary genital herpes infections are caused by HSV-II (Corey et al. 1986a).

Primary herpetic gingivostomatitis is a common oral condition that usually occurs in infants and children; however, it is not uncommon for teenagers and adults to present with the disease (Kerr 1952; Rees and Matheson 1986). The disease manifests itself as a severe stomatitis involving the entire oral cavity along with the pharnyx and perioral area. Prodromal symptoms precede the oral lesions, and include fever, irritability, malaise, headache, nausea, and occasional vomiting (Lynch et al. 1984). Submandibular and rarely cervical lymph nodes become enlarged and tender.

Approximately one to two days after the onset of prodromal symptoms, small fluid-filled vesicles appear thoughout the oral cavity. These quickly rupture leaving shallow, round, discrete ulcers. The patient also displays a generalized acute gingivitis which is manifested by extreme erythema and swelling. The infection is usually self-limiting and the oral lesions begin to heal within seven to ten days (Shafer et al. 1983; Lynch et al. 1984).

Primary genital herpes is characterized by fever, headache, malaise, and myalgia. Pain, itching, dysuria, vaginal and urethral discharge, and inguinal lymphadenopathy are the predominant local signs and symptoms (Corey et al. 1986b). The eruption of vesicles is observed on the external genitalia of both males and females often spreading to the thighs and buttocks (Adam 1982). Lesions tend to rupture, ulcerate, and form a crust. The lesions usually heal within two to four weeks without residual scarring.

During the primary infection, exposure of the virus at mucosal or abraded epidermal surfaces permits entry of virus into the host tissues and subsequent viral replication takes place. Whether or not clinically perceptible herpetic lesions develop, sufficient viral replication may occur to permit infection of either sensory or autonomic nerve endings (Corey et al. 1986a).

The virus then travels intra-axonally to the nerve-cell bodies in the ganglion. Openshaw, Puga, and Notkins in 1979 suggested that an acute infection developed when the virus reaches the ganglion. Infectious virus has been isolated in cell-free ganglionic homogenates (Openshaw et al. 1979) and trigeminal ganglia of human cadavers (Warren et al. 1978). Animal experiments have shown that antibody production as well as cell-mediated immunity is necessary to eliminate this acute infection (Openshaw et al. 1979).

Once the acute infection has been resolved, the latent stage of ganglionic infection begins. Two hypotheses have been postulated regarding viral latency. The virus may exist: (1) in a "static" non replicating state within the neurons, or (2) in a "dynamic" state where a low level of virus multiplication occurs continuously (Adam 1982; Corey et al. 1986a). Animal studies tend to support the first of these because infectious virus and viral antigens cannot be demonstrated during latency (Openshaw et al. 1979). Several investigators currently believe that the non-infective core of the viral genome remains undisturbed in the ganglionic neurons until reactivation of the virus takes place (Straus et al. 1985; Corey et al. 1986a).

Fever blisters, or cold sores as they are commonly known, are manifestations of recurrent oral herpes simplex infections. Even though 70-90% of the population harbors

antibodies to the virus, not all these persons have recurrences (Bader et al. 1978). The weight of evidence suggests that recurrent herpes is not a reinfection but rather a reactivation of a pre-existing virus in the host (Lynch et al. 1984). Recurrences appear to be, at least partly, under immunogenetic control; however, the mechanisms by which reactivation of the virus occurs is unknown (Wildy and Gell 1985). Various stimuli associated with reactivation are fever, anxiety, sunburn, trauma, or menstruation (Ship, Brightman, and Laster 1967). Reactivation of latent virus within the ganglia results in the transportation of viral genomes, in some unknown form, to the sites of recurrent outbreaks on the skin and mucous membranes (Corey et al. 1986b).

Recurrent HSV-I lesions are most often seen on the lips or perioral skin as "fever blisters", but may involve any part of the body. A prodromal period often precedes the onset of recurrent herpes labialis. Symptoms of the prodrome may include a burning, tingling, and/or swelling of the affected area. Shortly thereafter, one or a few erythematous papules form. These vesiculate, sometimes coalesce, and then break to form an often painful and disfiguring crust which generally last about 7 to 14 days, and heals without scarring.

Intraoral lesions may appear simultaneously with herpes labialis or as isolated lesions (Ficarelli, Tesini,

and Kane 1977a). The intraoral lesions typically appear as a cluster of small shallow ulcers. They tend to form most often on the hard palate and attached gingiva of affected individuals.

Recurrent genital herpes infections are almost always caused by HSV-II because primary genital infections caused by HSV-I tend not to recur (Corey et al. 1986b). Over one-half of the individuals who experience a primary genital attack suffer recurrent infections within one year (Straus et al. 1985). The lesions recur on the external genitalia of affected individuals and may also involve the vagina and cervix of females (Corey et al. 1986b). Recurrent episodes tend to have a milder course than the primary infections (Adam 1982).

Herpes simplex labialis is usually nothing more than an unsightly nuisance. The relief of pain and/or disfigurement are the primary concern of the otherwise healthy patient. However, herpetic infections of the oropharyneal and perioral areas in the immunocompromised patient represent a serious and potentially life-threatening event (Peterson and Sonis 1983). There is also a higher incidence of herpetic infections in these individuals as compared to immunologically healthy persons. The incidence has been found to be as high as 50% in patients undergoing bone marrow transplantation (Hann, Prentice, and Blacklock 1983). Herpetic lesions in

these individuals are usually multiple and often coalesce to form large ulcerations in the oral as well as perioral areas. The lesions may persist for three to four weeks. Recurrences appear to be very frequent in such patients with new episodes often developing before previous ones have completely healed. The risk of disseminated systemic involvement and secondary bacterial infection is a major consideration in the management of immunosuppressed patients.

A review of the literature reveals numerous studies describing various treatment modalities in the management of recurrent herpes simplex labialis (Blincoe and Miller 1979; McDonald et al. 1980). Despite the vast number of treatment methods, there still appears to be no definitive therapeutic regime that effectively reduces the severity of the lesions or lessens the healing time. The antiviral drug, acyclovir, has shown some promise in the topical treatment of these lesions; however, the results have been less than anticipated initially. Some authors have speculated that the ineffectiveness of acyclovir was due to lack of skin penetration of the drug (Gangarosa 1982; Spruance et al. 1984a). Various means have been used to enhance penetration but results have been mixed (Fiddian et al. 1983a; Shaw et al. 1985).

Iontophoresis has been shown to be an effective mechanism for enhancing skin penetration of drugs through

the use of ionic charges present on the drugs themselves (Gangarosa 1974; Lekas 1979; Gangarosa 1982). To date, only two studies have tested the effects of a topical application of acyclovir using iontophoresis. One study reported a favorable response to the therapy while the other showed little if any benefit. This study will attempt to provide evidence of the efficacy of iontophoretically applied acyclovir in the treatment of herpes simplex labialis.

# CHAPTER II

## REVIEW OF THE LITERATURE

The literature is replete with studies describing various treatments for herpes simplex labialis. The large majority of these reports are directed at a palliative treatment. The most popular remedies are not used to cure the lesions but simply to ease the patient's discomfort. Numerous over-the-counter preparations have been recommended. They include Blistex[1], Campho-Phenique[2], First-Aid Cream[3], Diaperene ointment[4], and A & D ointment[5] (McDonald et al. 1980). Topical anesthetic solutions, such as viscous lidocaine, have also been used for temporary relief of pain (Blank 1975).

---

[1]Blistex Incorporated, 1800 Swift Dr., Oak Brook, Il. 60521

[2]Winthrop Consumer Products, 90 Park Ave., N.Y., N.Y. 10016

[3]Johnson and Johnson Products, 501 George St., New Brunswick, N.J. 08903

[4]Glenbrook Laboratory, 90 Park Ave., N.Y., N.Y. 10016

[5]Schering Corporation, Galloping Hill Rd., Kenilworth, N.J. 07033

As mentioned previously the herpes simplex virus is surrounded by a lipid envelope (Hicks et al. 1979). This envelope is thought to play a major role in the infectivity of the virus; however, it is easily removed by lipid solvents <u>in vitro</u> (Corey et al. 1986a). Thus one approach to the treatment of herpes simplex labialis has been to use substances that can destroy this lipid membrane. Ethyl ether (Nugent and Chou 1973; Pasricha, Krishan, and Pasricha 1973), ethyl chloride (Blincoe et al. 1979), and chloroform (Taylor et al. 1977) are all lipid solvents that have been tried with mixed results. All of these substances have caused earlier crusting of the herpetic lesions; however, complete healing times have not been significantly reduced.

Cryotherapy with liquid nitrogen (Krashen 1970) and silver nitrate (Coleman et.al. 1973) have also been utilized. Both tend to inactivate the virus <u>in vitro</u> and may aid in patient comfort by destroying sensory nerve endings associated with the lesions. Other astringents of limited value due to their caustic nature are glutaraldehyde (Gordon 1976) and dinitrochlorobenzene (Jarratt, Smith, and Knox 1979).

Photoinactivation of the virus was very popular in the past. However, its carcinogenic potential has lead to its disuse (Bockstahler, Lytle, and Hellman 1975). The process involved painting a heterotricyclic dye, such as

neutral red, on the open lesion and then exposing the area to a fluorescent light source for approximately fifteen minutes (Felber, et al. 1973). These dyes have an affinity for the guanine in DNA and light exposure disrupts the nucleic acids within the virus (Berger and Papa 1977).

Repeated smallpox vaccinations showed promise in the past but currently are infrequently used due to limited availability of the vaccine. This treatment often resulted in a decrease in the frequency of herpetic recurrences (Kern and Schiff 1959; Lyon 1961). Current research aimed at perfecting a vaccine against the herpes simplex virus has shown promise in animal studies. Unfortunately for this vaccine to be effective it must be used in subjects that have never been exposed to the virus (Notkins et al. 1985).

Levamisol, a drug which is thought to increase cell mediated immunity, has also been tried with limited success (Russell, Brisson, and Grace 1978). The rationale for its use is that cellular immunity is thought to play a role in the resolution of recurrent herpetic lesions (Corey et al. 1986b). The use of intradermal gamma globulin has also been reported (Redman et al. 1986). The results in healing time, however, were no better than an intradermal histamine control.

Antibiotics do not affect viral replication, thus their use should be limited to the treatment of secondary bacterial infections (McDonald et al. 1980). However, the use of antiseptic compresses using benzalkonium or potassium permanganate have been reported to help alleviate patient discomfort (Adam 1982). The use of a supersaturated solution of aluminum potassium sulfate has shown to dry the vesicles and enhance healing (Hurt 1971). Topical corticosteroids have been suggested by some but are generally contraindicated due to possible dispersion of the viral infection over larger areas (McDonald et al. 1980).

Dietary additives, such as bioflavinoid-ascorbic acid and lysine, have been employed in the treatment of herpes simplex labialis. Bioflavinoid-ascorbic acid decreased the pain associated with the lesions and enhanced healing (Terezhalmy, Bottomley, and Pelleu 1978). Reports on lysine therapy have been mixed. Milman, Scheibel, and Jessen in 1978 reported that there was no difference in healing time or frequency of recurrence when the use of lysine was compared to a control. However, Thein and Hurt in 1984 tound a decrease in the frequency of occurrences as well as hastening of healing of the lesions.

Application of human interferon has also been reported in the treatment of herpes simplex infections

(Schmitt 1986). Interferon is actually a group of proteins derived from living cells in response to various stimuli (Stewart 1979). Several classes of interferon exist. Their anti-viral action is thought to be related to the formation of a new cellular RNA offsetting the viral RNA that has been encoded in the host cell (Richtmeier 1984). It has shown limited benefit in the treatment of herpes simplex labialis.

The bulk of the more recent research involves the use of anti-viral agents to disrupt the DNA of the replicating viruses. A purine nucleoside derivative, 9-B-D-arabinofuranosyladenine (ara-A, vidarabine, adenine arabinoside) has been shown to have antiviral properties against HSV I and HSV II. It may be useful in the treatment of neonatal HSV infections and herpes infections in immunocompromised patients (Chou 1984).

Five-iodo-2'-deoxyuridine (Idoxuridine, IDU) has shown mixed results in the treatment of herpes labialis. Kibrick and Katz, in 1970, found that neither the topical application of 0.5% IDU ointment or 0.1% IDU in polyvinyl alcohol had any therapeutic effect. However, MacCallum and Juel-Jenson, in 1966, reported an approximate 60% reduction in the healing time of herpes labialis with combinations of IDU and dimethyl sulphoxide (DMSO).

Several experimental antiviral agents have shown promise in the treatment of herpes infections.

2-deoxyglucose is thought to interfere with the synthesis of viral glycoproteins and success has been reported in the treatment of initial and recurrent genital herpes infections (Blough and Giuntoli 1979). Trisodium phosphonoformate (Alenius et al. 1982), E-5-(2-Bromoovinyl)-2'-deoxyuridine (De Clercq and Zhang 1982), ribavirin (Bierman, Kirkpatrick, and Fernandez 1981), and 2'-fluoro-5 iodoaracytosine (Lopez, Watanabe, and Fox 1980) have all been tested in vitro with success; however, toxic side effects have precluded their use in humans.

Acyclovir (9-<2=hydroxyethoxymethyl>guanine) is one of the newest of the approved anti-viral agents and currently the most promising (Chou 1984). It is 10- to 150-fold more potent in vitro than IDU or ara-A (Spruance, McKeough, and Cardinal 1984). Acyclovir has a high selective specificity for herpes simplex and varicella-zoster viruses with virtually no toxicity to host cells (McGuirt and Furman 1982). It is a structural analogue of the nucleoside guanine and is actively taken up by both virally infected and non-infected host cells (Bean 1983). Once inside the infected cell, acyclovir is phosphorylated to acyclovir monophosphate by a HSV-specific thymidine kinase enzyme. This in turn is phosphorylated to the active form, acyclovir triphosphate, by cellular enzymes of the host (Bean 1983).

Acyclovir triphosphate competitively inhibits viral DNA polymerase and terminates viral DNA replication by incorporating into the viral DNA chain (Elion 1982). The synthesis of host DNA in actively replicating uninfected cells in the presence of acyclovir is essentially unaffected because host-specific thymidine kinase cannot effectively phosphorylate acyclovir (Elion 1982). It has been postulated that HSV is inhibited by acyclovir at concentrations one thousand times less than those which would inhibit host cell proliferation (Chou 1984).

Acyclovir, in the United States, is available as an ointment for topical application, in capsules for oral administration, and as a sterile powder for intravenous infusion. It has been approved for use in the treatment of initial and recurrent genital herpes and HSV infections of all types in immunocompromised patients. Topical acyclovir significantly reduced the amount of viral shedding and time to complete crusting in primary genital herpes (Corey et al. 1982), but has shown little clinical benefit in the treatment of recurrent genital herpes infections (Reichman et al. 1983).

Oral acyclovir significantly reduced the amount of viral shedding and new lesion formation in the treatment of initial (Bryson et al. 1983) and recurrent genital herpes infections (Straus et al. 1984), but has shown limited clinical benefit in the treatment of recurrent

herpes labialis (Raborn et al. 1987). Oral administration of the drug has also shown a reduction in frequency of recurrent herpes genitalis when given prophylactically (Lemak, Duvic, and Bean 1986). Prophylactic intravenous use of acyclovir has been shown to significantly reduce the number of recurrences of HSV infections in bone marrow transplant recipients (Hann et al. 1983).

The results of topical acyclovir therapy in treating herpes simplex labialis have been mixed. Spruance and Crumpacker, 1982a, reported no clinical benefit from the use of 5% acyclovir in polyethylene glycol (PEG) ointment. However, that study and a subsequent one revealed that early therapy (within eight hours) resulted in a decrease in viral shedding (Spruance et al. 1982c). In a more recent study, the same authors have investigated early patient initiated treatment of herpes labialis with topical 10% acyclovir in polyethylene glycol (Spruance et al. 1984a). Despite the optimal timing of treatment, they reported no clinical benefit in the acyclovir-treated patients compared to placebo-treated patients.

In the United Kingdom and Europe where 5% acyclovir in a modified aqueous cream with 40% propylene glycol as a solvent has been the preferred topical formulation, the results of therapeutic studies in oral HSV infections have been in direct contrast to those in the United States.

Fiddian, in two separate studies, found that both 5% acyclovir-PEG and 5% acyclovir cream had promising clinical benefits. Acyclovir-PEG did not significantly decrease the duration of the lesions but did increase the number of abortive lesions when treated in the prodromal stage (Fiddian et al. 1983b).

Herpes labialis treated with 5% acyclovir cream showed a shortened time to onset of crusting and a decrease in total healing time when compared to a placebo cream of 40% propylene glycol (Fiddian et al. 1983a). A similar study found no clinical benefit from treatment with 5% acyclovir cream compared with the same placebo control; however, treatment with either preparation was better than no treatment (Shaw et al. 1985). The median healing time was 13 days when no treatment was given, 9 days when acyclovir cream was used, and 10 days when the placebo cream was used. The author suggested that the cream base alone may have a therapeutic effect. However, other investigators have refuted this assumption based on their studies (Kingsley, Yeo, and Fiddian 1985).

Assessment of the reasons for the clinical failure of topical acyclovir therapy has led to the conclusion that either therapy was initiated too late to alter the natural course of herpes labialis or that acyclovir inadequately penetrates the stratum corneum of the skin (Kinghorn 1985). Dimethyl sulfoxide (DMSO) has been

combined with 5% acyclovir and tested against experimental HSV-I infections. Spruance, McKeough, and Cardinal in 1982 showed that acyclovir in DMSO reduced the number of lesions developed on guinea pig skin by 80% following inoculation of HSV-I when compared to 5% acyclovir in PEG. A subsequent study by the same group in 1984 confirmed these results and also found a reduction in lesion size and virus shedding with acyclovir in DMSO. The penetration of acyclovir through guinea pig skin was measured and there was a marked increase in drug influx when acyclovir was combined with DMSO. This increased penetration of acyclovir may have accounted for the increase in effectiveness noted. However promising these results appear, treatment with DMSO in humans has not been approved by the United States Federal Drug Administration.

Another method of enhancing skin penetration of a drug is by means of iontophoresis (Lekas 1979). It is a safe and simple technique for promoting the penetration of a charged molecule into surface tissues (Gangarosa 1982). Iontophoresis has been used in dentistry for desensitizing hypersensitive dentin with flouride (Gangarosa 1982), for the treatment of aphthous ulcers with steroids (Gangarosa 1982), and to produce deep topical anesthesia with lidocaine and epinephrine. In 1974, Gangarosa reported that deciduous teeth could be extracted using

iontophoretically applied 2% lidocaine with 1/25,000 epinephrine.

A study by Hill, Gangarosa, and Park in 1977 indicated that skin penetration of anti-viral drugs can be enhanced by iontophoresis. Using neonatal mice as their model, they demonstrated that Ara-A, IDU, and phosphonoacetic acid (PAA) blocked neonatal skin DNA synthesis when administered by iontophoresis. There was a significantly greater effect when iontophoresis was used compared to topical application of the drugs alone. Since HSV-I is a DNA virus, it is reasonable to expect this mode of therapy would be effective in treating herpes simplex labialis.

In a preliminary clinical trial IDU was applied iontophoretically to oral herpetic lesions. Results were characterized by immediate relief of discomfort, rapid appearance and coalescence of vesicles, and accelerated healing with minimal crusting when compared to untreated controls (Gangarosa et al. 1979). In a letter to the editor, Boxhall and Frost in 1984 estimated that patients treated iontophoretically with IDU had up to 70 percent reduction in healing time of their herpetic lesions. However they used no controls and the results were based on patient assessment of healing as compared to their "usual" pattern of spontaneous healing.

A recent report using iontophoresis and anti-virals for the treatment of herpes labialis was published by Henley-Cohn and Hausfeld in 1984. They examined the clinical benefit of IDU and acyclovir. Both drugs when administered by iontophoresis produced a marked improvement in clinical symptoms. However, several deficiencies in the experimental design existed. First, there was no randomized, placebo-controlled design. The so-called placebo effect is a real phenomenon in patients with recurrent herpes labialis (Shaw et al. 1984). Second, imprecise measures were used to monitor the results. While some mention of clinical healing was reported, the patient's own assessment of therapy was the primary means of treatment analysis. Finally, only four patients were treated with acyclovir, a sample too small to provide meaningful assessment of treatment efficacy. All four patients, however, reported a major response from the treatment. Pain was relieved within six hours following treatment and all lesions were crusted and healing within twenty-four hours. This study suggests that topical application of acyclovir by iontophoresis could be an effective treatment modality for herpes labialis.

The most recent report involved both acyclovir and vidarabine monophosphate (ara-AMP). A placebo, NaCl, was also utilized. In this study, reported by Gangarosa et

al., in 1986, nine patients were included in each of the three treatment groups mentioned above. Each patient was treated only once with iontophoresis and the treatment medicament. Patients were seen frequently for up to 21 days and assessment of the healing of the herpetic lesions was monitored. The results indicated that there were no significant differences between healing response of the placebo and acyclovir. However, the ara-AMP group showed a significant decrease in duration of viral shedding and time to crust of the lesions. No difference was noted in overall healing time.

To further test the efficacy of these antiviral agents in combination with iontophoresis, "therapeutic trials" were conducted on large cutaneous lesions of herpes simplex and herpes zoster (Gangarosa et al. 1988). Lesions were treated and re-examined at twenty-four hours for signs of improvement. The results suggested that Ara-AMP and acyclovir were both equally effective in the treatment of the herpes zoster lesions; however, the Ara-AMP was more effective than acyclovir in the treatment of the herpes simplex lesions. Both were significantly more effective than the placebo.

The differences obtained in these last two reports were possibly due to changing the treatment electrode from the cathode to the anode during some of the "therapeutic trials" (Gangarosa, 1987). However, positive results were

obtained with both treatment electrodes. The conflicting nature of the results of these two reports combined with the positive implications of Henley-Cohn (1984) indicates a further need to study the effects of iontophoretically applied acyclovir.

# CHAPTER III

## MATERIALS AND METHODS

Patients for this study were actively solicitated from Baylor College of Dentistry and the Baylor Medical Complex. All participants were volunteers who either responded to bulletin-board announcements (Appendix 1), were referred by persons familiar with the study, or were subjects who had participated in a previous study and indicated a desire to be included in the present study. Thirty-five patients were screened using a standard set of criteria (Appendix 2). Of these, twenty-five patients were selected and these agreed to participate in the study.

Patient selection was based upon medical history, frequency of herpetic recurrences, and patient willingness to participate. The patients were otherwise healthy individuals who gave a history of at least three episodes of circumoral herpetic lesions in the past twelve months. Pregnant females, patients with pace-makers, and patients with hepatitis, AIDS, venereal disease, or other communicable diseases were excluded.

The majority of the initial screenings were performed over the telephone. Once accepted for the

study, patients were told to contact the investigator at the earliest evidence of a recurrent episode. Baseline data (Appendix 3), to include a health history questionnaire (Appendix 4), were collected at the initial visit of these individuals. Several other participants, reporting to the clinic in person, were added to the study population after the initial screening period. These individuals were interviewed and, if accepted, they completed the initial baseline data and health history at the screening appointment. All participants were required to sign a written consent (Appendix 5).

A total of sixteen subjects began the study. They were randomly divided into two study groups (Group A and B). Additional subjects were added evenly to these groups as they presented. The final groups consisted of thirteen (Group A) and twelve (Group B) individuals. The participants remained in these groups and received the same treatment throughout the study.

The trial was conducted as a double-blind, placebo-controlled study. The test material, acyclovir (Zovirax[1]), was obtained in capsule form. Each capsule contained 200 mg of acyclovir. The placebo, sodium bicarbonate, was selected based on similarities

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[1]Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709

with acyclovir in color, consistency, and appearance in solution. The placebo was placed in an identical capsule as the acyclovir. Capsules were then placed in separate vials labeled either Group A or B by a non-participating clinician.

The patients were instructed to contact the investigator as soon as they were aware of a developing lesion. All lesions were treated within twenty-four hours of onset. If a patient presented after twenty-four hours of onset the lesion was followed untreated. Also, if a patient presented with multiple lesions at the same time, one lesion was left untreated. Thus, a third group consisting of untreated lesions was established.

At the initial appointment, excluding the screening appointment, a cytologic smear was obtained from the herpetic lesion. The lesion was washed with sterile saline and dried. The lesion was then gently teased open with a sterile #15 bard parker blade. Vesicular fluid was adsorbed on a sterile cotton swab and placed on a labeled glass slide. The slide was subsequently stained using a Papanicolaou stain, and viewed at a later date for typical HSV cytopathologic effects: intranuclear inclusions (Lipshutz bodies), multinucleated epithelial cells, and ballooning degeneration which is often seen with a perinuclear halo (Nowakovsky 1968). Some of these changes can be seen in the photomicrographs presented (Fig. 1-4).

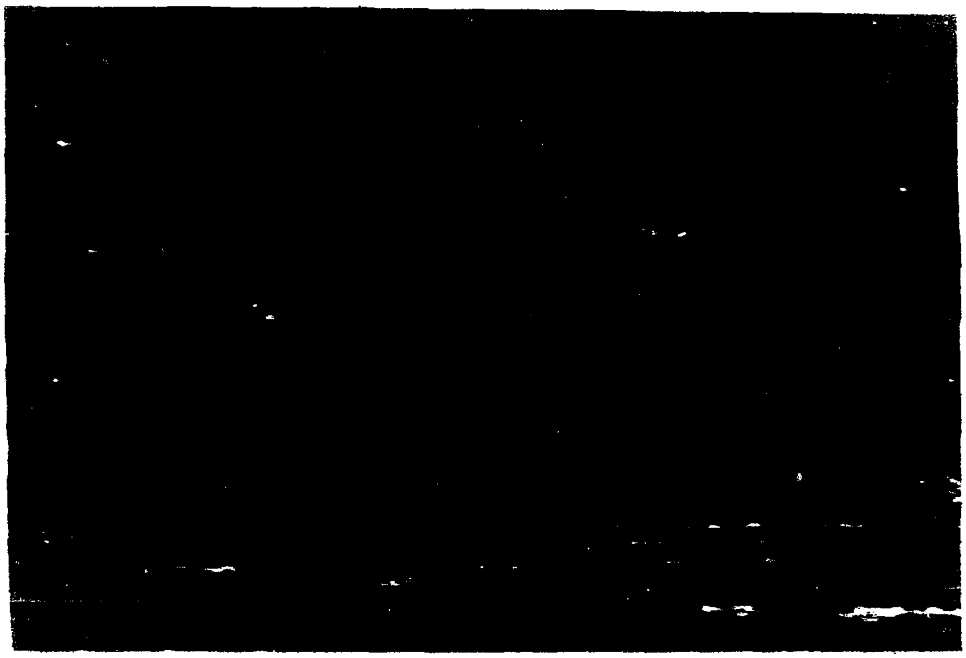

FIGURE 1.--Cytology of two normal epithelial cells. Note the polyhedral cellular morphology with a small, centrally located nucleus (Magnification X 132).

FIGURE 2.--Early viral alterations include a tendency for the epithelial cells to clump and also form lobulations within the nucleus (Magnification X 66).

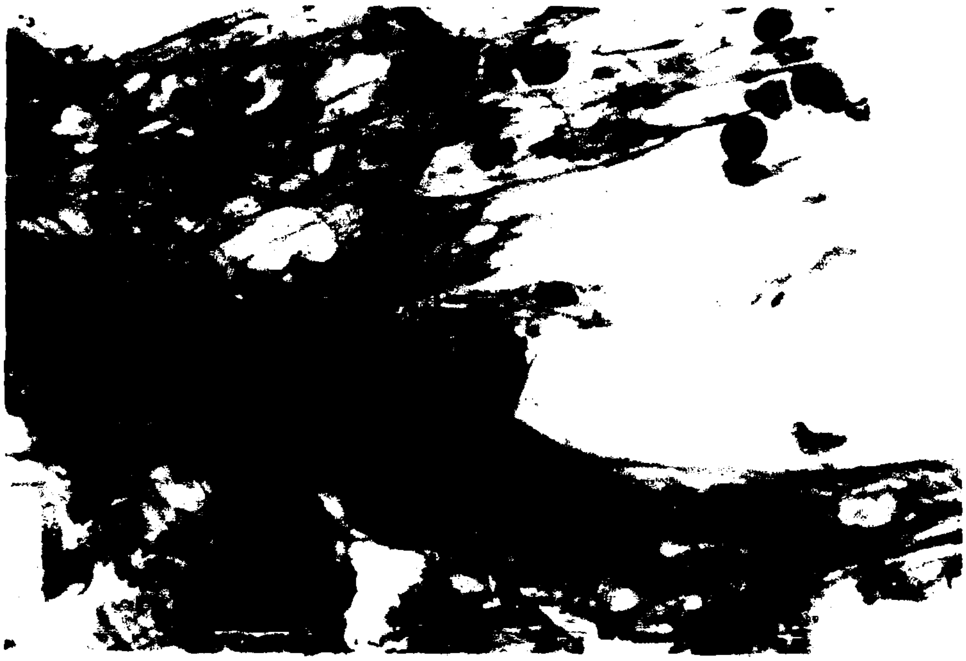

FIGURE 3.--Note a virally infected epithelial cell showing a ballooned nucleus with a perinuclear halo. A second smaller epithelial cell can be seen with the nucleus displaced to one side (Magnification X 132).

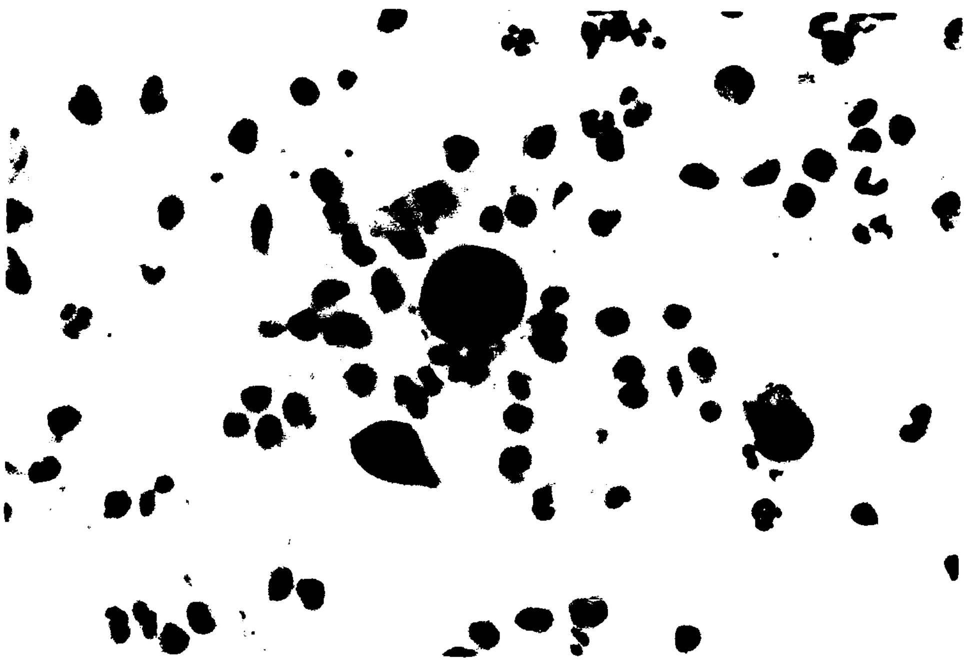

FIGURE 4.--Note two epithelial cells with multilobulated nuclei giving the cells the appearance of "multinucleation". Intranuclear eosinophilic material present is viral DNA. Also note numerous inflammatory cells (mostly neutrophils) in the field (Magnification X 132).

Information was obtained from the patient regarding the onset of each occurrence and individual episode (Appendix 6). The lesions were then classified as to size, location, stage, and presence or absence of pain (Appendix 7). This procedure was also repeated at each followup appointment. The size of each lesion was measured using a standard millimeter ruler in the horizontal and vertical plane across its widest part. The lesions were staged according to the following: erythema, papule, vesicle, crust, absence of crust, and complete healing of the lesion.

The treatment consisted of a single iontophoretic application of either acyclovir or the placebo. A capsule was removed from the bottle corresponding to the patient group and mixed with 10 ml of sterile demineralized water in a clear beaker. A cotton roll was cut into sections and placed into the solution. Varying sizes of applicator attachments were available (Fig. 5). An attachment was selected that not only covered the entire lesion, but also overlapped the lesion margins by approximately two millimeters. A corresponding section of the soaked cotton roll was then placed in the plastic attachment by means of a sterile cotton pliers.

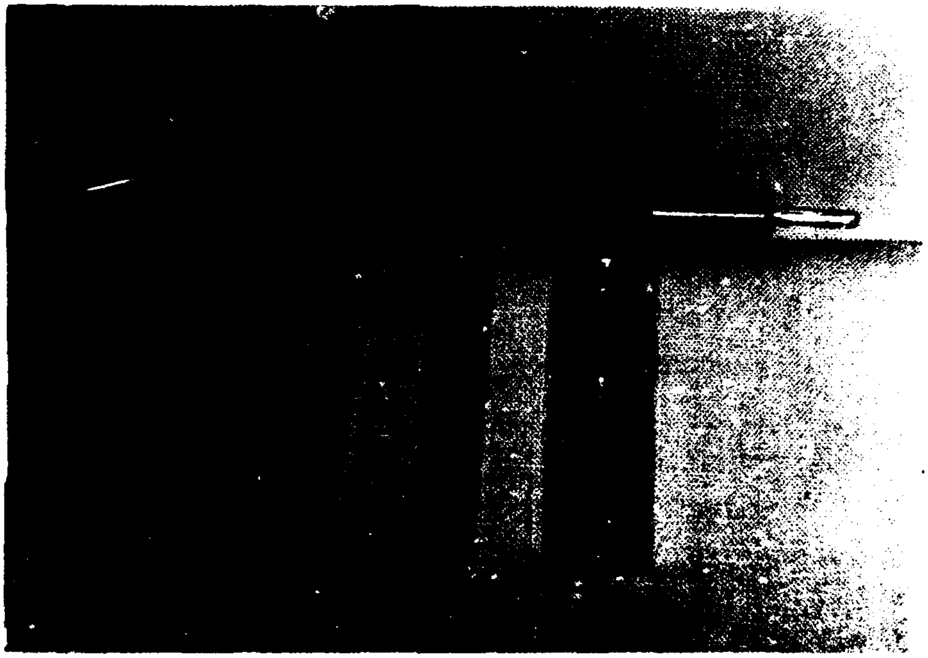

FIGURE 5.--Varying sizes of plastic attachments are available for use with the treatment applicator. During the study this applicator was connected to the positive electrode of the iontophoresis unit.

The iontophoresis unit used in the study was the Dentelect ElectroApplicator[1] (Fig. 6). The treatment applicator was connected to the positive electrode (anode) of the iontophoresis unit. The plastic attachment with cotton was then applied to the lesion by the patient holding constant pressure over the area (Fig. 7). The return electrode (cathode) included an adhesive bandage-like device consisting of a paper disk saturated with sodium nitrate. This was attached to the volar surface of the patient's midforearm (Fig. 8). The current on the machine was allowed to run at 0.4 - 0.8 mA for 5 - 10 minutes resulting in an electrical charge flow of 4.0 mA-minutes. After treatment, the subject was asked to keep the lesion as dry as possible and avoid undue irritation to the area.

The healing phase of the lesions was monitored at subsequent visits. All patients returned the first day following treatment. The subjects were also seen for two or three more visits at varying time intervals until the lesions were completely healed. Complete healing was defined as the loss of all visible evidence of a previous lesion. At all visits the lesions were photographed, measured, and staged as previously indicated. All

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[1] ElectroApplicator System Model C-2, Dentelect Corporation, Augusta, Ga.

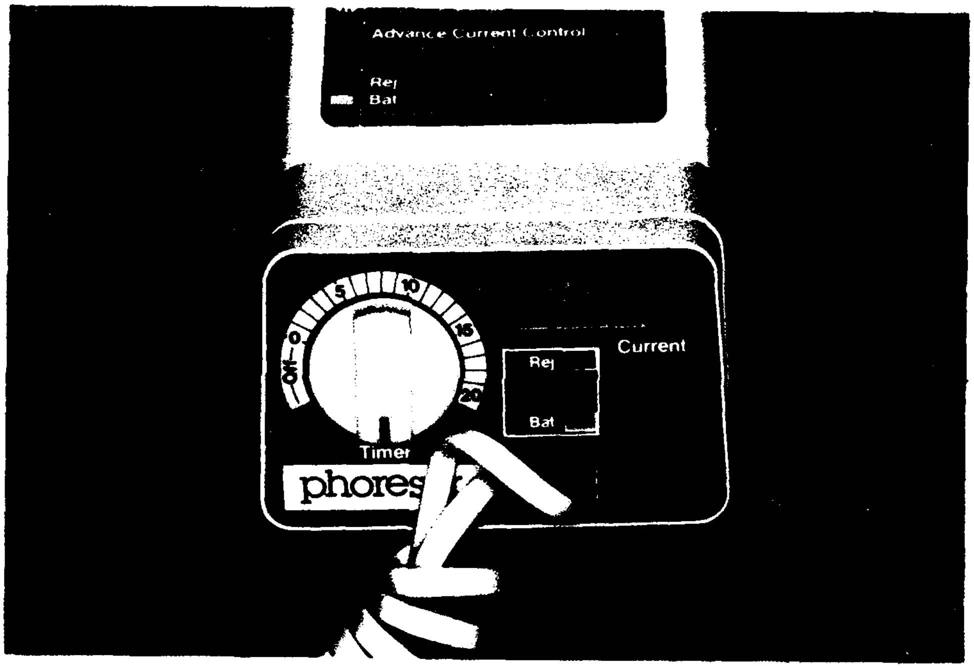


FIGURE 6.--The Denelect ElectroApplicator used in the study provided the current for the iontophoresis procedure.

FIGURE 7.--The iontophoretic application as it was performed during the study. Note that the patient holds the applicator to ensure constant and equal pressure over the lesion.

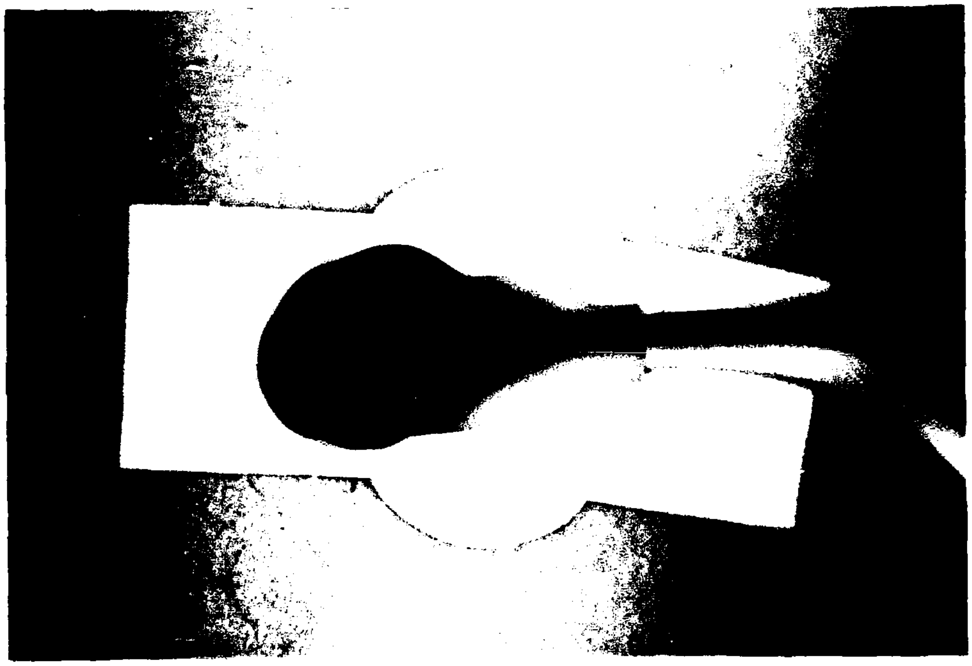

FIGURE 8.--The return electrode was attached to the volar surface of the patient's forearm and connected to the negative electrode of the iontophoresis unit.

subsequent episodes were similarly treated. After completion of the study, patient assessment of the efficacy of the treatment as compared to previous treatment modalities was reported (Appendix 8).

Statistical analysis of the data included descriptive statistics, a one-way ANOVA test, and Chi square. Descriptive statistics were used to report the patient's assessment of the treatment. Data on the effectiveness of treatment in regard to healing time were analyzed using Chi square. The analysis of variance test was used to evaluate the difference in lesion size between the groups.

# CHAPTER IV

# RESULTS

A total of twenty-five participants was included in the study. As shown in Table 1, there were 13 individuals in group A and 12 individuals in group B. The experiment was conducted as a double-blind, placebo controlled study. Throughout the study, group B received treatment with the test material, acyclovir; whereas, group A received treatment with the placebo. A smaller third group which consisted of non-treated lesions was also established. This group contained individuals from both groups A and B and included a total of six patients.

Of the 25 participants included in the study, only 11 actually reported for treatment with any herpetic lesions. Of these, seven were in group A and four were in group B. The untreated group included lesions from three patients in group A and three patients in group B. One of the four patients reported in group B was only included in the untreated group because her only reported lesion was beyond 24 hours. Thus only three patients actually received treatment with acyclovir. The total number of individuals and lesions reported for each group can be seen in Table 1.

TABLE 1. -- Number of Individuals and Number of Lesions

| Group | Total Number of Individuals | Individuals with Lesions | Number of Lesions |
|---|---|---|---|
| Acyclovir | 12 | 4 | 18 |
| Placebo | 13 | 7 | 14 |
| Untreated | | (6)* | 10 |

*3 patients from each of the other two groups.

Throughout the study, lesion dimensions were determined by measuring the lesion in a horizontal and vertical plane across its widest part. These measurements were then multiplied to give an area for each lesion which was reported in square millimeters. A one-way ANOVA test was used to compare the initial size of the lesions (prior to treatment) among the three groups. This statistical test was also used to evaluate differences in lesion area between groups following treatment. Tables 2, 3, and 4 contain cumulative data collected during the study related do lesion size and duration.

Analysis of other data refealed that when present, the most frequent prodromal symptoms reported were tingling, burning, and swelling. However, one third of the reported lesions were without prodromal symptoms (Table 5). Precipitating factors associated with the lesions included fever, exposure to sunlight, anxiety, and dental treatment. Of these, fever and exposure to sunlight were most often cited by the participants (Table 6). A summary of the data on lesion locadion, shown in Table 7, reveals that the lesions were reported mobe frequently in the midline or to the left of the midline. When combining the number of lesions found on the skin under the nose with those of the upper lip an equal distribution between those reported on the upper lip vs. those on the lower lip is found.

TABLE 2.--Summary of Data (Acyclovir)

| Patient Number | Size mm$^2$ (day 0) | Size mm$^2$ (day 1) | Days to Loss of Crust | Days to Complete Healing |
|---|---|---|---|---|
| 1 | 20 | 30 | 6 | 11 |
| 1 | 49 | 42 | 7 | 11 |
| 1 | 16 | 16 | 8 | 11 |
| 1 | 40 | 30 | 6 | 9 |
| 4 | 6 | 4 | 5 | 7 |
| 4 | 16 | 36 | 6 | 9 |
| 4 | 42 | 25 | 4 | 8 |
| 4 | 12 | 16 | 3 | 6 |
| 4 | 48 | 48 | 9 | 14 |
| 4 | 120 | 132 | 9 | 15 |
| 4 | 42 | 56 | 11 | 15 |
| 4 | 25 | 25 | 5 | 9 |
| 4 | 6 | 6 | 4 | 9 |
| 4 | 9 | 9 | 8 | 11 |
| 4 | 12 | 9 | 7 | 10 |
| 4 | 24 | 20 | 9 | 14 |
| 6 | 25 | 36 | 7 | 10 |
| 6 | 20 | 16 | 3 | 7 |

TABLE 3.--Summary of Data (Placebo)

| Patient Number | Size $mm^2$ (day 0) | Size $mm^2$ (day 1) | Days to Loss of Crust | Days to complete Healing |
|---|---|---|---|---|
| 5 | 25 | 16 | 8 | 11 |
| 5 | 36 | 36 | 9 | 12 |
| 7 | 42 | 54 | 7 | 14 |
| 7 | 30 | 30 | 8 | 12 |
| 7 | 8 | 9 | 6 | 9 |
| 7 | 54 | 90 | 9 | 12 |
| 8 | 56 | 80 | 4 | 7 |
| 9 | 9 | 6 | 6 | 8 |
| 9 | 9 | 12 | 5 | 9 |
| 10 | 4 | 6 | 7 | 11 |
| 10 | 16 | 20 | 8 | 12 |
| 10 | 12 | 12 | 5 | 9 |
| 11 | 40 | 48 | 5 | 10 |
| 12 | 8 | 9 | 4 | 8 |

TABLE 4.--Summary of Data (Untreated)

| Patient Number | Size $mm^2$ (day 0) | Size $mm^2$ (day 1) | Days to Loss of Crust | Days to Complete Healing |
|---|---|---|---|---|
| 1 | 63 | 88 | 9 | 15 |
| 2 | 16 | 20 | 11 | 15 |
| 4 | 20 | 30 | 11 | 14 |
| 4 | 20 | 25 | 8 | 13 |
| 4 | 20 | 20 | 9 | 14 |
| 5 | ? | 30 | 10 | 14 |
| 7 | 30 | 36 | 9 | 11 |
| 7 | 16 | 36 | 13 | 17 |
| 9 | 9 | 9 | 5 | 7 |
| 9 | ? | 66 | 10 | 15 |

TABLE 5.--Prodromal Signs and Symptoms

| Itch | Tingle | Burning | Swelling | None |
|---|---|---|---|---|
| 4 | 8 | 9 | 9 | 14 |

TABLE 6.--Precipitating Factors

| Fever | Exposure to Sunlight | Dental Treatment |
|---|---|---|
| 15 | 9 | 4 |
| Anxiety | None | |
| 5 | 10 | |

TABLE 7.--Lesion Location

| Midline | Right | Left | Lower Lip | Upper Lip |
|---|---|---|---|---|
| 13 | 9 | 19 | 19 | 14 |
| Under the Nose | Commissure | | | |
| 7 | 1 | | | |

The statistical analysis performed on the initial lesion dimensions revealed that there was no statistical difference between any of the groups regarding lesion size prior to treatment (Table 8). Further analysis revealed that there was no statistical difference found between the groups at the one day follow-up (Table 9). Thus one can assume that the groups were equal at the initiation of treatment as regards to overall lesion size. The data also indicate that there was no difference in lesion size between groups A and B following treatment. There were also no differences between these two groups and the third untreated group regarding lesion size at the one day follow-up.

A Chi-square analysis was used to determine if there were any differences in healing time between the three groups (Tables 10 and 11). There was no statistical difference between groups A and B as to the time the crust was lost or time of complete healing of the lesions. However, there was a statistical difference (.01 level) between group A and the untreated group as to the time until loss of the crust. There was also a statistical difference (.05 level) between group A and the untreated group as regards time to complete healing. Group B was also statistically different (.01 level) from the untreated group as regards time to loss of crust and time to complete healing of the lesions.

TABLE 8.--Size Prior to Treatment

| Group | Number of lesions | Mean |
|---|---|---|
| Acyclovir | 18 | $29.56mm^2$ |
| Placebo | 14 | $24.93mm^2$ |
| Untreated | 8 | $24.25mm^2$ |

ONE-WAY ANOVA

| Source | SS | DF | MS | F |
|---|---|---|---|---|
| Factor 1 | 237.50 | 2 | 118.75 | .24* |
| Error | 18526.3 | 37 | 494.29 | |

*No Statistical Difference

TABLE 9.--Size at Day One Followup

| Group | Number of lesions | Mean |
|---|---|---|
| Acyclovir | 18 | $30.89mm^2$ |
| Placebo | 14 | $30.57mm^2$ |
| Untreated | 8 | $33.00mm^2$ |

ONE-WAY ANOVA

| Source | SS | DF | MS | F |
|---|---|---|---|---|
| Factor 2 | 33.19 | 2 | 16.60 | .02* |
| Error | 28517.2 | 37 | 770.74 | |

*No Statistical Difference

TABLE 10.--Time to Loss of Crust

| Group | (3-7 days) | (8-13 days) |
|---|---|---|
| Acyclovir | 12 | 6 |
| Placebo | 9 | 5 |
| Untreated | 1 | 9 |

CHI SQUARE ANALYSIS OF THE DATA

1. Acyclovir/Placebo--No Significant Difference
CHI SQ = .02
DF = 1

2. Acyclovir/Untreated--Significant Difference P<.01
CHI SQ = 8.29
DF = 1

3. Placebo/Untreated--Significant Difference P<.01
CHI SQ = 7.09
DF = 1

TABLE 11.--Time to Complete Healing

| Group | (7-11 days) | (12-17 days) |
|---|---|---|
| Acyclovir | 13 | 5 |
| Placebo | 9 | 5 |
| Untreated | 2 | 8 |

CHI SQUARE ANALYSIS OF THE DATA

1. Acyclovir/Placebo--No Significant Difference
   CHI SQ = .23
   DF = 1

2. Acyclovir/Untreated--Significant Difference $P<.01$
   CHI SQ = 7.06
   DF = 1

3. Placebo/Untreated--Significant Difference $P<.05$
   CHI SQ = 4.6
   DF = 1

Tables 12 and 13 contain cumulative data concerning the patient's own assessment of the treatment received during the study. One patient in group B reported only once with a lesion which had been present beyond 24 hours, and thus the lesion was included in the untreated group. All three of the patients in group B who received treatment reported a faster healing time when compared to previous treatment modalities. One of the patients also reported a decrease in lesion severity. Four out of the seven patients treated in group A also indicated a benefit in healing time when compared to previous treatment modalities. However, three of the seven patients indicated no difference in efficacy of treatment. With the exception of one patient who showed no improvement, all patients who had both treated and untreated lesions showed an enhanced healing time when comparing treated lesions to untreated regardless of which treatment was employed.

TABLE 12.--Efficacy of Treatment Based on Patient Assessment

| Group | Improved | Worse | No Change |
|---|---|---|---|
| Acyclovir | 3(100%) | -- | -- |
| Placebo | 4(57%) | -- | 3(43%) |

TABLE 13.--Efficacy of Treatment Related to Improvement

| Group | Decreased Frequency | Decreased Severity | Decreased Duration |
|---|---|---|---|
| Acyclovir | -- | 1(33%) | 3(100%) |
| Placebo | -- | -- | 4(57%) |

# CHAPTER V

# DISCUSSION

Of the 25 subjects who were included in the study, only 11 actually reported for treatment with any herpetic lesions. The remaining 14, or 56% of the original volunteers, reported no lesions during the entire study period of one year. This overall lack of response can possibly be explained in several ways. First, the patients could have over-estimated the number of lesions which occurred over the previous year. A more probable explanation would be a lack of compliance on the part of the participants during the study period.

Over half of the total number of subjects included in the study were participants in another recent clinical trial involving the treatment of herpes labialis. Only two of these subjects reported lesions during this study while all of them reported lesions during the previous study. For whatever reason, this group of patients appeared to be unwilling to report for treatment in the present study. The requirement for several followup visits with each lesional episode may also have discouraged participation.

Of the 11 patients who did report lesions, seven received treatment with the placebo. Four patients in group B reported lesions; however, only three actually received treatment with acyclovir. One patient in this group had a lesion reported in the untreated group. A total of three patients from each group were included in this third group of untreated lesions. Even though the number of patients treated in each group was small, the overall number of lesions per group was great enough to perform meaningful statistical analysis.

Lesion dimension was determined at each treatment and post-treatment visit. As seen in Table 8, the pre-treatment mean size within the acyclovir group was greater than the other two groups; however, statistical analysis revealed that there was no statistical difference between any of the three groups regarding initial lesion size. One day following treatment in the acyclovir and placebo groups the mean lesion size was approximately the same while the mean of the untreated group was larger. The mean lesion size of all three groups at the one day follow-up appointment had increased slightly when compared to the pre-treatment sizes. The untreated group showed the largest change in lesion size; however, there was no statistical difference regarding change in lesion size among any of the groups.

As seen in Tables 2, 3, and 4, there was a large variation in individual lesion size between subjects as well as within the came subject. With the exception of one lesion in the acyclovir group, all lesions were less than 63 $mm^2$ at the initial appointment. There was a tendency for larger lesions to plateau in size over the first few days while the smaller lesions tended to reach a peak in size within the first 24 hours. In a study examining the natural history of recurrent herpes labialis, Spruance et al. (1977) reported that median lesion size tended to reach the maximum within the first 24 hours and then decline progreccively thereafter. In the present study, the increase in lesion size initially followed by a subsequent reduction in size appears to reflect the natural healing process reported by Spruance.

The most frequent prodromal signs and symptoms reported in this study were tingling, burning, and swelling. However, as seen in Table 5, 14 lesions (33%) were reported without prodromal symptoms. Two studies dealing with the natural history of recubrent herpetic lesions found similar results. Bader et al. in 1978 found that 76% of patients with recurrent episodes had prodromal symptoms; while Spruance et al. (1977) reported that 85% of the patients had such findings. In both studies the major prodromal symptoms reported were itching, tingling, burning, and pain.

Table 6 reveals data related to precipitating factors of the lesions. Fever and exposure to sunlight were most often reported by the subjects. One patient stated that all of his lesions occurred approximately 24 hours following dental treatment. Seventy-seven percent of the episodes had at least one precipitating factor associated with its occurrence. In close correlation, it was reported that 76% of the patients in a previous study also indicated an association with a predisposing factor (Bader et al. 1978). In this later study, the most frequent factors listed were upper respiratory tract infections, fatigue, and emotional stress.

The lesions were most often located on the lips (83%). The upper and lower lips tended to be equally affected as has been reported in previous studies (Spruance et al. 1977; Bader et al. 1978). Bader also stated that no preferential area could be found on either lip. To the contrary, the present study found that most lesions (79%) occurred in the midline or to the left of the midline.

The three parameters that appear to be most beneficial in assessing the efficacy of treatment when studying herpes simplex labialis are: (1) measurements of virus titer, (2) time until loss of scab or crust and (3) time to complete healing (Overall 1982). Spruance et al. (1977) reported that mean virus titers reached a peak at

24 hours during the natural healing of untreated lesions. Virus titers were generally higher in vesicular lesions, however, conversion of a lesion from a vesicle to a ulcer/crust was not necessarily associated with a sharp drop in viral titer. No virus could be isolated after five days. Gangarosa et al., in 1986, reported a significant reduction in virus titer after 24 hours when vidarabine monophosphate was applied iontophoretically as compared with lesions that were treated with iontophoretically applied acyclovir or the NaCl placebo. However, there was no significant difference in healing time between the treatment groups.

The procedure for obtaining a virus titer for comparison involves opening and swabbing the lesions every 24 hours. It seems logical that this procedure itself would alter the healing of the lesions. Therefore, the present study assessed treatment efficacy by analyzing time to the loss of the crust and time to complete healing. Tables 10 and 11 reveal that there were no differences between the lesions treated with acyclovir and the placebo in regards to both of these healing parameters. This is in agreement with a previous study in which acyclovir was compared to a placebo (NaCl) and another antiviral agent, vidarabine monophosphate (Gangarosa et al. 1986).

Gangarosa (1986) reported a mean time to complete healing of 11.7 days for the lesions treated with acyclovir and 11.6 days for those treated with the placebo. The present study found that there was a mean time to complete healing of 10.6 days for the acyclovir group and 10.2 days for the placebo group. The mean time to loss of crust in the present study was 7.0 days for the lesions treated with acyclovir and 6.5 days for the lesions treated with the placebo. This data was not included in the Gangarosa study.

One of the interesting findings of the present study was that there was a significant difference in healing time between the untreated lesions and both of the other groups. The mean time to loss of crust for the untreated lesions was 9.5 days. The mean time to complete healing for this group was 13.5 days. Thus there was an approximate delay in healing time of three days for the untreated lesions when compared to the lesions treated with the placebo or acyclovir.

Of the ten lesions reported in the untreated group there were five from patients in the acyclovir group and five from patients in the placebo group. When evaluated separately, the five lesions in the acyclovir group had a mean time to complete healing of 10.6 days when treated and 14.2 days when untreated. The five lesions in the placebo group had a mean time to complete healing of 10.9

days when treated and 12.8 days when untreated. This data may suggest that there is a trend toward enhanced healing with acyclovir; however, lesion numbers were too small to analyze the data statistically.

Bader et al., in 1978, reported a mean healing time of 9.3 days for the natural healing of lesions left untreated. However, these lesions were manipulated each day as previously described for assessment of virus titer. This procedure may have resulted in an enhanced healing time by reducing the amount of virus present. The criteria used for complete healing in Bader's study is not comparable with that in the present study. The present study defined complete healing as the loss of all visible evidence of a previous lesion. Bader used categories of "nearly healed" and "healed" without a description of either.

There are several possible explanations for the enhanced healing found in the two treated group of lesions. The first is a true placebo effect in which lesions of patients who received any treatment (placebo or acyclovir) healed faster than those which were not treated. This so-called placebo effect has been shown to play a role in previous studies involving recurrent herpetic lesions (Russell et al. 1978; Overall 1982). The desire of the patients to find anything that can relieve them of the stigma of these lesions causes an apparent

beneficial effect to be derived from seemingly inert placebo controls.

To further support this, the subjective data collected at the end of the study concerning the patient's own assessment of the treatment indicated that the majority of patients felt that the treatment had resulted in significant improvement over previous treatments. All of the patients treated in the acyclovir group and 4 out of 7 (57%) treated in the placebo reported improvement. All of the patients in both groups combined who reported improvement stated that there was a decrease in lesion duration. One patient also reported a decrease in severity of the lesions.

The placebo effect may not be entirely psychological. Shaw et al., in 1985, reported on a study involving the treatment of herpes labialis with 5% acyclovir cream and a placebo control. The median healing times were 13 days when no treatment was given, 9 days when acyclovir cream was used, and 10 days when the placebo cream was used. The author suggested that there could be a therapeutic effect from the placebo cream alone.

Taking this into consideration, other possible explanations for the findings in the present study exist. The beneficial effect from the placebo may be related directly to the sodium bicarbonate. Sodium bicarbonate

has been demonstrated to possess antibacterial properties (Newburn, Hoover, and Ryder 1984). It is possible that it may also effect viral activity by some undetermined mechanism. The positive results may also have been produced by the electric current generated during the iontophoresis process. To further evaluate the later possibility, another group consisting of lesions treated with acyclovir and the iontophoresis unit without active current should be added to the study.

So far, none of the studies involving topical acyclovir have reported a decrease in lesion frequency. Thus the present study was not designed to evaluate this parameter. Of the patients who reported improvement over previous treatment modalities, none reported a decrease in lesion frequency. To evaluate this parameter effectively, a preliminary evaluation period of at least six months and preferably one year would be required.

All patients were treated within 24 hours of lesion onset. Even so, most lesions were fully developed at the time of initial presentation. If not in the vesicle stage, lesions were visibly evident by erythema and/or swelling. Light microscopy reveals that even at the vesicular stage the underlying intact epithelial cells already show extensive viral damage (Cawson 1986). Thus these cells are not likely to be restored to normal by anitviral the.apy. Lesions should be treated in the

prodrome to provide optimum benefit to the patient (Spruance and Wenerstrom 1984). Unfortunately, this period is brief and presents a major obstacle to the design of clinical trials.

In the present study, no side-effects due to the acyclovir were noted. However, several patients reported a mild stinging sensation during the iontophoresis process. This was reported at the lesion site as well as at the return electrode site on the patient's forearm. When this condition was untolerable to the patient, the current was decreased on the ElectroApplicator. This should not affect the treatment of the lesion because the decrease in current was offset by an increase in treatment time resulting in a consistent amount of mA-minutes.

# CHAPTER VI

## SUMMARY OF RESULTS

Of the 25 patients who were registered in the study, only 11 actually reported for treatment with any herpetic lesions. Of these, seven were in the placebo group and four were in the acyclovir group. Of these four only three actually received treatment with acyclovir. A third group of untreated lesions consisted of lesions from three patients in each of the other two groups.

Analysis of the data revealed that initial mean lesion size was similar in all three groups and that there was no significant difference in lesion size between the groups one day following treatment. Prodromal symptoms and precipitating factors associated with the lesions closely paralleled those described in previous studies. An unusual finding was that the majority of lesions were located in the midline or to the left of the midline of the patient's vermilion or perioral skin.

Analysis of the data related to lesion duration revealed that there was no significant difference between the placebo and acyclovir groups. However, there was a statistically significant enhancement in healing found in both treated groups when compared to the untreated

lesions. The patient's own assessment of the treatment revealed that all of the patients in the acyclovir group and four of the seven patients in the placebo group noticed improvement over previous treatment modalities utilized. All patients reporting improvement indicated a decrease in lesion duration.

# CHAPTER VII

## CONCLUSIONS

1. There was no significant difference between the iontophoretic application of acyclovir and a placebo (sodium bicarbonate) regarding the duration of herpetic lesions. However, both demonstrated some efficacy when compared to untreated lesions.

2. It appears that under these experimental conditions acyclovir is not clinically beneficial in the treatment of oral herpetic lesions. However, future studies involving other antiviral agents and iontophoresis may be warranted.

3. Patients who have recently been involved in a clinical trial may not be good candidates for a second study.

APPENDIX 1

Bulletin-Board Announcement

RESEARCH VOLUNTEERS NEEDED

Persons suffering from FEVER BLISTERS or COLD SORES are asked to participate in a research study. A topical application of medication will be utilized. Anyone interested, please contact Dr. Lewis Humphreys, Department of Periodontics, Baylor College of Dentistry, at 828-8146.

APPENDIX 2

## Cursory Patient Selection Criteria

Name: ______________________________ Age: ____

Address: ______________________________________

Telephone: (home) __________ (work) ______________

Today's date: __________

1. Medical problems: ____________________________

2. Currently being treated by a physician? ________

If yes, then why? ______________________________

3. Current medications: _________________________

4. Pregnant? ________ Pace-maker? _______________

5. Hepatitis, venereal disease, AIDS? ____________

6. Number of fever blisters in past year? ________

7. Will you agree to the following?

yes no

___ ___ a. Sign an informed consent?

___ ___ b. Not use any other treatment method for the herpetic lesions during the study.

___ ___ c. Contact Dr. Humphreys at the first onset of a lesion (within 24 hours) and return for three subsequent visits?

___ ___ d. Allow a specimen to be taken from the initial lesion.

___ ___ e. Allow photographs to be taken at each appointment.

APPENDIX 3

Baseline Data

Name: ______________________ Sex: __ Age: ___

Address: ______________________________

Telephone: __________ (wk), __________ (hm)

Employment: ______________________

Todays Date: ____________

1. Frequency of herpetic lesions ______________

2. Duration of lesions ____________________

3. Location of lesions ____________________

______________________________________

4. Usually single or multiple ________________

5. Describe prodromal symptoms if present ______

______________________________________

6. List previous treatments _________________

______________________________________

7. Results of treatments ___________________

______________________________________

8. Describe a typical episode ________________

______________________________________

APPENDIX 4

## Medical History

Name: ________________________________

Age: ____ Sex: ____ Ht.: ____ Wt.: ____ Race: ____

Occupation: ________________ Martial status: __

Physician,address,phone #: ____________________

________________________________________

Date of last physical exam: _____ Reason: __________

________________________________________

Are you presently being treated by a physician?: _____

Why?: ________________________________

Are you taking any medication?: ____ Why?: ________

________________________________________

To the best of your knowledge, have you ever had or do you now have any of the following:

Cardiovascular system:

Yes/No Heart trouble

Yes/No Heart murmur

Yes/No Rheumatic fever

Yes/No Swollen or painful joints

Yes/No Prolonged fever

Yes/No High or low blood pressure

Yes/No Shortness of breath

Yes/No Frequent nose bleeds

Yes/No Stroke

Appendix 4--Continued

Endocrine System:

Yes/No Thyroid condition, goiter

Yes/No Diabetes

Yes/No Gland problems

Yes/No Members of your family with diabetes

Respiratory System:

Yes/No Respiratory disease

Yes/No Asthma, hayfever, or allergies

Yes/No Tuberculosis

Yes/No Chronic cough, hoarseness, or sore throat

Yes/No Do you smoke

Gastrointestinal System:

Yes/No Stomach or intestinal trouble

Yes/No Frequent indigestion, diarrhea, or vomiting

Yes/No Difficulty in swallowing

Yes/No Jaundice or Hepatitis

Yes/No Liver disease

Genitourinary System:

Yes/No Kidney disease

Yes/No Frequent urination

Yes/No Swollen ankles

Yes/No Any reaction of medications, if so what? _____

______________________________________________________

Appendix 4--Continued

Nervous System:

Yes/No Mental disorders

Yes/No Epilepsy or convulsions

Yes/No Neuritis, neuralgia or numbness

Blood:

Yes/No Blood diseases

Yes/No Dizziness or fainting spells

Yes/No Anemia

Bones and Joints:

Yes/No Excess bleeding from cut or tooth extraction

Yes/No Arthritis or rheumatism

Yes/No Frequent bone fractures

Yes/No Any condition requiring steroid therapy

Others:

Yes/No Tumors, cysts, cancer

Yes/No Recent gain or loss in weight

Yes/No Major operations

Yes/No Pregnancy or menstrual trouble

Yes/No Skin rash, hives

Yes/No Venereal disease

Yes/No Mumps

Yes/No Measles

Yes/No Chicken pox

APPENDIX 5

## Informed Consent

In order to evaluate the effectiveness of a topically applied antiviral agent, acyclovir, in the treatment of recurrent herpes simplex labialis, I voluntarily consent to its application or the application of a placebo as described below. Acyclovir cream will be applied topically by the researcher using a machine that emits a small electrical charge. This charge enhances the agent's ability to penetrate the skin.

I understand that as far as can be ascertained by reviewing the results of earlier investigations using similar agents and techniques, apart from a mild, tingling sensation at the site of application, no other side effects have been observed. I agree to notify Dr. Humphreys if any unusual symptoms develop during the course of the study.

Benefits of the study include the possibility that a new treatment modality for herpes simplex infections will be found that shortens the healing time and decreases the severity of the lesions.

The welfare of each participant will be protected during the study. I understand that my right to withdraw from this study at any time is fully assured without penalty.

Appendix 5--Continued

I authorize the taking of necessary diagnostic records (i.e. clinical measurements and photographs) and understand that such records may be used for reproduction in scientific publications or for teaching purposes and that my identity will not be revealed.

In addition to allowing the administration of the topical agent, I agree to the following:

(1) Call Dr. Humphreys for an appointment immediately upon initial symptoms of a lesion. I also agree to followup visits 3-5 days and 8-10 days after the first visit;

(2) Allow a swab specimen to be taken for viral typing;

(3) Not to employ any other treatment measures against the herpes lesions during the testing period;

(4) To complete and sign a medical history form;

(5) To complete a questionaire concerning the herpes episode at each appointment.

After carefully reading this information, and having had my participation in the study thoroughly explained to me, with the opportunity to have questions answered, I hereby willingly consent to participate in this study on the use of a topically applied antiviral agent against recurrent herpes simplex infections,

Appendix 5--Continued

conducted under the auspices of Baylor College of Dentistry. In case I need to contact someone I can call Dr. Humphreys at 828-8126. I understand that the institutional contact is Dr. Frazier (phone 828-8321).

Date:

Signed:

Witness:

# APPENDIX 6

## Patient Questionnaire

1. Date and time lesion or prodromal symptoms were initially noticed. ______________________

2. If present, describe prodromal symptoms. __________

______________________________________________

3. If present, describe lesion pain. __________

______________________________________________

4. Can you attribute any stimuli to the initiation of the lesion? (i.e. sunlight, trauma, fever, emotional anxiety, or menstrual period.) __________

______________________________________________

5. At present are you taking any medication? __________

______________________________________________

APPENDIX 7

## Clinical Assessment

Lesion Data Date: __________

1. Location ______________________________
2. Size ______________________________
3. Stage ______________________________
4. Pain (yes/no) ______________________________

Lesion Data Date: __________

1. Location ______________________________
2. Size ______________________________
3. Stage ______________________________
4. Pain (yes/no) ______________________________

Lesion Data Date: __________

1. Location ______________________________
2. Size ______________________________
3. Stage ______________________________
4. Pain (yes/no) ______________________________

APPENDIX 8

## Assessment by the Patient

1. How do you feel about the effects of the treatment?

    (a) It helped

    (b) Lesions were worse

    (c) No detectable change

2. If it helped, how did it help?

    (a) Decreased the severity of the lesions.

    (b) Shortened the duration of the lesions.

    (c) Decreased the recurrence rate.

3. Did you have any lesions that were not reported to the investigator?

    No___ Yes___ (How many? _____)

Did you use any other medications to treat the lesions during the course of the study?

    No___ Yes___ (What medications?)_______________

_______________________________________________

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CURRICULUM VITAE

PERSONAL INFORMATION:

Name: Lewis Gilbert Humphreys, Jr.
Birthplace: Chattanooga, Tennessee
Birthdate: March 3, 1954
Marital Status: Married (Alicia)
Children: Ryan, Mikelyn, Colleen
Military Service: United States Air Force 1982-present

EDUCATION:

| | |
|---|---|
| Brainerd High School<br>Chattanooga, Tn. | 1968-1971 |
| Auburn University<br>Auburn, Al.<br>Degree: B.S. Biology | 1971-1975 |
| UAB School of Dentistry<br>Birmingham, Al.<br>Degree: D.M.D. | 1976-1980 |
| Baylor College of Dentistry<br>Dallas, Tx.<br>Certificate in Periodontics<br>(Candidate for M.S. Degree) | 1986-1988 |

HONORS:

Auburn University -graduated with High Honor, 1976.
UAB School of Dentistry - Annual Award in Periodontics, 1980.

PROFESSIONAL SOCIETIES:

American Dental Association, 1980-present
American Academy of Periodontology, 1986-present
Southwest Society of Periodontics, 1986-present

LICENSURE:

June 1980 Georgia State Dental Board
June 1980 Alabama State Dental Board

ORAL PRESENTATIONS:

October 1987
Comprehensive Case Report (Faculty and Graduate Students All Disciplines)

April 1988
Periodontal Case Report (Annual Texas Periodontal Resident's Day)

May 1988
Periodontal Case Report (Sophomore Dental Students)

May 1988
Comprehensive Case Report (Faculty and Graduate Students of All Disciplines)